

We claim:

- 1 1. A plasmid comprising:
2 a primer sequence capable of annealing to a first portion of nucleic acid
3 encoding a polypeptide;
4 a collar sequence capable of annealing to a second portion of the nucleic acid
5 encoding a polypeptide, the second portion of the nucleic acid encoding a polypeptide
6 being at least 20 nucleotides removed from the first portion of the nucleic acid
7 encoding a polypeptide; and
8 at least one restriction site located between the primer and collar sequences.
- 1 2. A plasmid as in claim 1 wherein the primer and collar sequences are capable of
2 annealing to first strand cDNA encoding a polypeptide.
- 1 3. A plasmid as in claim 1 wherein the primer and collar sequences are capable of
2 annealing to mRNA encoding a polypeptide.
- 1 4. A plasmid as in claim 1 wherein the primer and collar sequences are capable of
2 annealing to mRNA encoding at least a portion of an antibody.
- 1 5. A plasmid as in claim 1 wherein the collar sequence is capable of annealing to
2 a portion of the nucleic acid encoding a polypeptide that is remote in the 5' direction
3 from the portion of the nucleic acid to which the primer sequence is capable of
4 annealing.
- 1 6. A host cell transformed with a plasmid of claim 1.
- 1 7. A method of producing nucleic acid encoding a polypeptide, the method
2 comprising the steps of :

1 a) providing a plasmid containing a primer sequence adapted to anneal to a
2 first portion of nucleic acid encoding a polypeptide and a collar sequence adapted to
3 anneal to a second portion of the nucleic acid encoding a polypeptide, the second
4 portion of the nucleic acid encoding a polypeptide being at least 20 nucleotides
5 removed from the first portion of the nucleic acid encoding a polypeptide;

6 b) cleaving the plasmid at one or more restriction sites, the one or more
7 restriction sites being located on the plasmid between the primer and collar
8 sequences; and

9 c) exposing the cleaved plasmid to nucleic acid encoding a polypeptide in an
10 aqueous environment containing nucleotides and one or more enzymes selected from
11 the group consisting of polymerase, reverse transcriptase, ligases and combinations
12 thereof to form a nucleic acid strand that is complementary to the nucleic acid
13 encoding a polypeptide, the complementary nucleic acid strand being formed between
14 the primer and collar sequences whereby the plasmid is ligated and circularized.

15 8. The method of claim 7 further comprising the step of removing the
16 complementary nucleic acid strand from the plasmid.

17 9. A method as in claim 7 wherein the step of cleaving the plasmid comprises
18 exposing the plasmid to an oligonucleotide that hybridizes to the one or more
19 restriction sites on the plasmid prior to exposing the plasmid to a restriction enzyme.

20 10. A method as in claim 7 wherein the step of exposing the cleaved plasmid to a
21 nucleic acid encoding a polypeptide comprises exposing the cleaved plasmid to mRNA
22 encoding a polypeptide.

23 11. A method as in claim 10 wherein the cleaved plasmid is exposed to mRNA
24 encoding at least a portion of an antibody.

1 12. A method as in claim 7 wherein the step of exposing the cleaved plasmid to a
2 nucleic acid encoding a polypeptide comprises exposing the cleaved plasmid to first
3 strand cDNA.

1 13. A host cell transformed with a circularized plasmid produced in step c) of claim
2 7.

1 14. A polypeptide produced using the plasmid of claim 1.

1 15. A polypeptide produced using the circularized plasmid produced in step c) of
2 claim 7.

1 16. A plasmid comprising:
2 a primer sequence adapted to anneal to a first portion of a nucleic acid
3 encoding a polypeptide;
4 a collar sequence adapted to anneal to a second portion of the nucleic acid
5 encoding a polypeptide, the second portion of the nucleic acid encoding a polypeptide
6 being at least 20 nucleotides removed from the first portion of the nucleic acid
7 encoding a polypeptide; and
8 a nucleic acid strand that is complementary to the nucleic acid encoding a
9 polypeptide, the complementary nucleic acid strand being formed in situ between the
10 primer and collar sequences.

1 17. A plasmid as in claim 16 wherein the primer and collar sequences are adapted
2 to anneal to first strand cDNA encoding a polypeptide.

1 18. A plasmid as in claim 16 wherein the primer and collar sequences are adapted
2 to anneal to mRNA encoding a polypeptide.

1 19. A plasmid as in claim 16 wherein the primer and collar sequences are adapted
2 to anneal to a portion of mRNA encoding at least a portion of an antibody.

- 1 20. A host cell transformed with a plasmid of claim 16.
- 1 21. A polypeptide produced using a plasmid of claim 16.
- 1 22. A plasmid containing a nucleic acid sequence encoding at least a portion of a
2 polypeptide prepared in accordance with the method of claim 7.
- 1 23. A plasmid comprising:
2 a downstream primer capable of annealing to a first portion of mRNA encoding
3 at least a portion of an antibody;
4 an upstream collar sequence capable of annealing to a second portion of the
5 mRNA encoding at least a portion of an antibody; and
6 at least one restriction site located between the downstream primer and
7 upstream collar sequence.
- 1 24. A plasmid as in claim 23 wherein the upstream collar sequence is capable of
2 annealing to a portion of the mRNA encoding a framework region of an antibody.
- 1 25. A plasmid as in claim 23 wherein the upstream collar sequence is capable of
2 annealing to a leader sequence of the mRNA encoding an antibody.
- 1 26. A plasmid as in claim 23 wherein the upstream collar sequence is capable of
2 annealing to a portion of the mRNA encoding a framework region associated with a
3 light chain of an antibody.
- 1 27. A plasmid as in claim 23 wherein the upstream collar sequence is capable of
2 annealing to a portion of the mRNA encoding a framework region associated with a
3 heavy chain of an antibody.
- 1 28. A plasmid as in claim 23 wherein the downstream primer is capable of
2 annealing to a portion of the mRNA encoding a constant region of an antibody.

1 29. A plasmid as in claim 23 wherein the downstream primer is capable of
2 annealing to a portion of the mRNA encoding a constant region associated with a light
3 chain of an antibody.

1 30. A plasmid as in claim 23 wherein the downstream primer is capable of
2 annealing to a portion of the mRNA encoding a framework two (FR2), framework three
3 (FR3) or framework four (FR4) region associated with a light chain of an antibody.

1 31. A plasmid as in claim 23 wherein the downstream primer is capable of
2 annealing to a portion of the mRNA encoding a constant region associated with a
3 heavy chain of an antibody.

1 32. A plasmid as in claim 23 wherein the downstream primer is capable of
2 annealing to a portion of the mRNA encoding a framework two (FR2), framework three
3 (FR3) or framework four (FR4) region associated with heavy chain of an antibody.

1 33. A plasmid as in claim 23 wherein the downstream primer comprises SEQ. ID.
2 NO: 4.

1 34. A plasmid as in claim 23 wherein the downstream primer comprises SEQ. ID.
2 NO: 8.

1 35. A plasmid as in claim 23 wherein the upstream collar sequence comprises
2 SEQ. ID. NO: 3.

1 36. A plasmid as in claim 23 wherein the upstream collar sequence comprises
2 SEQ. ID. NO: 7.

1 37. A host cell transformed with a plasmid of claim 23.

38. A method of producing nucleic acid encoding at least a portion of an antibody, the method comprising the steps of :

a) providing a plasmid containing a downstream primer adapted to anneal to a first portion of mRNA encoding at least a portion of an antibody and an upstream collar sequence adapted to anneal to a second portion of the mRNA encoding at least a portion of an antibody;

b) cleaving a plasmid at one or more restriction sites, the one or more restriction sites being located on the plasmid between the downstream primer and upstream collar sequence;

c) exposing the cleaved plasmid to the mRNA encoding at least a portion of an antibody in an aqueous environment containing reverse transcriptase and nucleotides to form a nucleic acid strand that is complementary to the mRNA, the complementary nucleic acid strand being formed between the downstream primer and upstream collar; and

d) ligating the complementary nucleic acid strand to the upstream collar whereby the plasmid is ligated and circularized.

39. The method of claim 38 further comprising the step of removing the complementary nucleic acid strand from the plasmid.

40. A method as in claim 38 wherein the step of cleaving the plasmid comprises exposing the plasmid to a restriction enzyme.

41. A method as in claim 40 wherein the step of cleaving the plasmid comprises exposing the plasmid to an oligonucleotide that hybridizes to the one or more restriction sites on the single strand plasmid prior to exposing the plasmid vector to a restriction enzyme.

42. A method as in claim 38 wherein the step of exposing the cleaved plasmid to the mRNA forms a complementary nucleic acid strand encoding at least a light chain of an antibody.

43. A method as in claim 38 wherein the step of exposing the cleaved plasmid to the mRNA forms a complementary nucleic acid strand encoding at least a heavy chain of an antibody.

44. A method as in claim 39 wherein the step of removing the complementary nucleic acid strand from the plasmid comprises digesting the plasmid containing the complementary nucleic acid strand.

45. A method as in claim 44 wherein the step of digesting the plasmid containing the complementary nucleic acid strand comprises the steps of hybridizing a oligonucleotide to a portion of the single strand antibody DNA to provide a double stranded restriction site and exposing the double stranded restriction site to a restriction enzyme.

46. A method as in claim 38 wherein the step of providing a plasmid comprises providing a plasmid wherein the upstream collar sequence is adapted to anneal to a portion of the mRNA encoding a framework region of an antibody.

47. A method as in claim 38 wherein the step of providing a plasmid comprises providing a plasmid wherein the upstream collar sequence is adapted to anneal to a portion of the mRNA encoding a framework region associated with a light chain of an antibody.

48. A method as in claim 38 wherein the step of providing a plasmid comprises providing a plasmid wherein the upstream collar sequence is adapted to anneal to a portion of the mRNA encoding a framework region associated with a heavy chain of an antibody.

49. A method as in claim 38 wherein the step of providing a plasmid comprises providing a plasmid wherein the downstream primer is adapted to anneal to a portion of the mRNA encoding a constant region of an antibody.

1 50. A method as in claim 38 wherein the step of providing a plasmid comprises
2 providing a plasmid wherein the downstream primer is adapted to anneal to a portion
3 of the mRNA encoding a constant region associated with a light chain of an antibody.

1 51. A method as in claim 38 wherein the step of providing a plasmid comprises
2 providing a plasmid wherein the downstream primer is adapted to anneal to a portion
3 of the mRNA encoding a constant region associated with a heavy chain of an
4 antibody.

1 52. A method as in claim 38 wherein the step of providing a plasmid comprises
2 providing a plasmid wherein the upstream collar sequence comprises SEQ. ID. NO: 3.

1 53. A method as in claim 38 wherein the step of providing a plasmid comprises
2 providing a plasmid wherein the upstream collar sequence comprises SEQ. ID. NO: 7.

1 54. A method as in claim 38 wherein the step of providing a plasmid comprises
2 providing a plasmid wherein the downstream primer comprises SEQ. ID. NO: 4.

1 55. A method as in claim 38 wherein the step of providing a plasmid comprises
2 providing a plasmid wherein the downstream primer comprises SEQ. ID. NO: 8.

1 56. A host cell transformed with a circularized plasmid produced in step d) of claim
2 38.

1 57. An antibody or antibody fragment produced using the plasmid of claim 23.

1 58. An antibody or antibody fragment produced using the circularized plasmid
2 produced in step d) of claim 38.

1 59. A plasmid comprising:

1 a downstream primer adapted to anneal to a first portion of mRNA encoding at
2 least a portion of an antibody;
3 an upstream collar sequence adapted to anneal to a second portion of the
4 mRNA encoding at least a portion of an antibody; and
5 a nucleic acid strand that is complementary to the mRNA, the complementary
6 nucleic acid strand being formed in situ between the downstream primer and upstream
7 collar sequence to close the plasmid.

1 60. A plasmid as in claim 59 wherein the upstream collar sequence is adapted to
2 anneal to a portion of the mRNA encoding a framework region of an antibody.

1 61. A plasmid as in claim 59 wherein the upstream collar sequence is adapted to
2 anneal to a portion of the mRNA encoding a framework region associated with a light
3 chain of an antibody.

1 62. A plasmid as in claim 59 wherein the upstream collar sequence is adapted to
2 anneal to a portion of the mRNA encoding a framework region associated with a heavy
3 chain of an antibody.

1 63. A plasmid as in claim 59 wherein the downstream primer is adapted to anneal
2 to a portion of the mRNA encoding a constant region of an antibody.

1 64. A plasmid as in claim 59 wherein the downstream primer is adapted to anneal
2 to a portion of the mRNA encoding a constant region associated with a light chain of
3 an antibody.

1 65. A plasmid as in claim 59 wherein the downstream primer is adapted to anneal
2 to a portion of the mRNA encoding a constant region associated with a heavy chain of
3 an antibody.

1 66. A plasmid as in claim 59 wherein the upstream collar sequence comprises
2 SEQ. ID. NO: 3.

1 67. A plasmid as in claim 59 wherein the upstream collar sequence comprises
2 SEQ. ID. NO: 7.

1 68. A plasmid as in claim 59 wherein the downstream primer comprises SEQ. ID.
2 NO: 4.

1 69. A plasmid as in claim 59 wherein the downstream primer comprises SEQ. ID.
2 NO: 8.

1 70. A host cell transformed with a plasmid of claim 59.

1 71. An antibody or antibody fragment produced using the plasmid of claim 59.

1 72. A plasmid containing a nucleic acid sequence encoding at least a portion of an
2 antibody prepared in accordance with the method of claim 38.

1 73. A plasmid as in claim 1 wherein two restriction sites that are the same or
2 different are located between the downstream primer and upstream collar sequences.

1 74. A plasmid as in claim 23 wherein two restriction sites that are the same or
2 different are located between the downstream primer and upstream collar sequence.

1 75. A library containing a repertoire of antibodies or antibody fragments made using
2 the plasmid of claim 23.

1 76. A library containing a repertoire of antibodies or antibody fragments made using
2 the method of claim 38.

1 77. A library containing a repertoire of antibodies or antibody fragments made using
2 the plasmid of claim 59.

1 78. A method of producing nucleic acid encoding a polypeptide, the method
2 comprising the steps of :

3 a) providing a plasmid containing a primer sequence adapted to anneal to a
4 first portion of nucleic acid encoding a polypeptide;

5 b) cleaving the plasmid at one or more restriction sites, the one or more
6 restriction sites being located on the plasmid to provide a cleaved plasmid having the
7 primer at one end thereof and a free end; and

8 c) exposing the cleaved plasmid to nucleic acid encoding a polypeptide in an
9 aqueous environment containing nucleotides and one or more enzymes selected from
10 the group consisting of polymerase, reverse transcriptase, ligases and combinations
11 thereof to form a nucleic acid strand that is complementary to the nucleic acid
12 encoding a polypeptide, the complementary nucleic acid strand being formed attached
13 to the primer and having a free end.

1 79. A method as in claim 78 further comprising the step of joining the free end of
2 the complementary nucleic acid strand to the free end of the plasmid whereby the
3 plasmid is circularized.

1 80. A method as in claim 79 wherein the step of joining comprises providing a
2 bridging oligonucleotide.

1 81. The method of claim 78 further comprising the step of removing the
2 complementary nucleic acid strand from the cleaved plasmid.

1 82. A method as in claim 78 wherein the step of exposing the cleaved plasmid to a
2 nucleic acid encoding a polypeptide comprises exposing the cleaved plasmid to first
3 strand cDNA.

1 83. A method as in claim 82 wherein the cleaved plasmid is exposed to antibody
2 cDNA encoding at least a portion of an antibody.

1 84. A method as in claim 82 wherein the first strand cDNA is cleaved at a restriction
2 site prior to being exposed to the cleaved plasmid.